Chemical Name: Afidopyropen
USEPA PC Code: 026200
USEPA MRID: 49689236
USEPA DP Barcode: 435146
PMRA Data Code: 9.2.4.6
PMRA Study No. (UKID): 2627508
Data Requirement (Guideline): OECD Guidance Doc. No. 75

Test Material: BAS 440 00 I (TEP, VERSYS™) **Purity:** 9.7%

Active Ingredient: Afidopyropen
IUPAC Name: [(3*S*,4*R*,4a*R*,6*S*,6a*S*,12*R*,12a*S*,12b*S*)-3-(cyclopropylcarbonyloxy)-1,2,3,4,4a,5,6,6a,12a,12b-decahydro-6,12-dihydroxy-4,6a,12b-trimethyl-11-oxo-9-(3-pyridyl)-11*H*,12*H*-benzo[*f*]pyrano[4,3-*b*]chromen-4-yl]methylcyclopropane carboxylate
CAS Name: [(3*S*,4*R*,4a*R*,6*S*,6a*S*,12*R*,12a*S*,12b*S*)-3-(cyclopropylcarbonyl)oxy)]-1,3,4,4a,5,6,6a,12,12a,12b-decahydro-6,12-dihydroxy-4,6a,12b-trimethyl-11-oxo-9-(3-pyridyl)-2*H*,11*H*-naphtho[2,1-*b*]pyrano[3,4-*e*]pyran-4-yl]methyl cyclopropanecarboxylate
CAS No.: 915972-17-7
Synonyms: INSCALIS™

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Date: 15 February 2018

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Date: 7 September 2017

Date Evaluation Completed: 7 September 2017

CITATION:
Alshcer, A., C. Claßen, and J. Staffel. 2015. Semi-field brood study to evaluate potential effects of BAS 440 00 I on brood development of honeybees (*Apis mellifera* L.). RIFCON GmbH, Goldbeckstraße 13, 69493 Hirschberg, Germany. Report No. 1440041. Sponsor: BASF SE. Report No. BASF Reg. Doc. #: 2015/1001368. USEPA MRID 49689236. PMRA UKID 2627508.

Executive Summary:
The semi-field (tunnel) study tested the effects of the formulated end-use product BAS 440 00 I (9.7% afidopyropen) on honeybee (*Apis mellifera*) colonies with the intent of examining brood (*i.e.*, eggs, larvae, pupae) development and colony strength (number and condition of adult bees/brood and available food reserves). The study design was based in part on OECD Guidance Document No. 75.

Nucleus bee colonies (containing 8,574 ± 297[1] adult bees/colony) within individual enclosures (108m^2 of which 84 m^2 cropped) containing phacelia (*Phacelia tanacetifolia*) in full bloom were exposed by foliar applications from a ground-boom sprayer, while bees were actively foraging, to either 0.50 L/ha (50 g a.i./ha; 0.04 lbs a.i./A) of BAS 440 00 I, the insect growth regulator fenoxycarb (300 g a.i./ha), or a water (negative) control treatment. During treatment, colonies were covered with plastic sheets to protect them from direct spraying.

Each treatment group consisted of four replicate tunnels[2], each containing a single nucleus colony; colonies were acclimated to the tunnels seven days before treatment. In addition to the four replicate tunnels in control and afidopyropen-treatment groups, there was an extra tunnel in each group used solely for residue monitoring. Colonies were maintained in tunnels for a total of 8 days after treatments (0-7 DAT, "exposure period"), and then transferred roughly 6 km to a remote monitoring site without a bee-attractive flowering crop for 20 days (8-27 DAT, "monitoring period"). Adult and larval/pupal mortality were recorded from three days before, to 26 days after treatments (-3 to 26 DAT). Assessments also included foraging activity (-3 to 7 DAT), colony condition (food stores, brood status, and colony strength) and bee brood development at 2, 8, 12, and 19 DAT. Treatment rates were not confirmed analytically and are therefore based on nominal treatment levels.

The preliminary brood check indicated healthy colonies with all brood stages present (8,455 ± 482 larvae/colony; 14,309 ± 579 pupae/colony), and a sufficient supply of nectar (15,400 ± 919 cells/colony) and pollen (1,764 ± 273 cells/colony). Throughout the post-application study period, the number of brood or food cells did not differ statistically among the three treatment groups. The mean numbers of bees per colony in the three treatment groups one day before application (-1 DAT) were similar. There were no statistically significant differences in adult worker bee mortality (68.33 ± 4.18 dead bees/colony), worker bee foraging activity (7.67 ± 0.87 bees/m^2), or pupal mortality (0.12 ± 0.05 dead pupae/colony) between the treatment groups before application.

Afidopyropen treatments resulted in significantly ($p < 0.05$) different (*i.e.*, lower) foraging activity during the exposure period, and significantly ($p < 0.05$) different (*i.e.*, higher) pupal mortality during the post-exposure monitoring period, relative to controls. Afidopyropen treatments also resulted in significantly ($p < 0.05$) different (*i.e.*, lower) adult worker bee mortality during the exposure and monitoring periods relative to control treatments; however, since mortality in the afidopyropen-treated colonies was lower than that in the negative control, it was not considered an adverse effect. While no sublethal behavioral effects were reported in control tunnels, afidopyropen treatments resulted in sublethal behavioral effects (*i.e.*, "coordination problems") for roughly 60 bees in the hours immediately following applications.

Results Synopsis:

[1] Note that all means in this summary are followed by ± one standard error (SE).
[2] Test item treatment tunnel no. 4 was excluded from the study after it was accidentally treated with the insecticide dimethoate, which was being used as a reference toxicant for a "parallel study".

The study is generally consistent with OECD Guidance Document No. 75, although there are some potentially important study deviations and deficiencies. Due to unusually high adult worker bee mortality (71.83 ± 5.07 dead bees/colony/d) during the pre-application period in control colonies, and adverse weather conditions throughout the study, the power of the study to detect treatment effects may be limited. As treatment levels were not analytically verified in the study, and due to possible effects of weather two days after applications, there is uncertainty regarding actual afidopyropen exposure levels.

Honey bee colonies treated with formulated afidopyropen at 50 g a.i./ha (0.04 lbs a.i./A) exhibited significant ($p<0.05$) adverse effects on foraging activity and pupae survival, resulting in a no-observed adverse effect level (NOAEL) of <50 g a.i./ha under the conditions tested. By the conclusion of the study, while there were adverse effects on pupae survival, there were no significant adverse effects on brood development in afidopyropen-treated colonies relative to control colonies, and adult honeybee mortality was lower in afidopyropen-treated colonies compared to control colonies.

EPA Classification: Supplemental (should only be used qualitatively)
PMRA Classification: Reliable with restrictions

I. DATA SOURCE

USEPA MRID No.:	49689236
PMRA UKID No.:	2627508
Study Title:	Semi-field brood study to evaluate potential effects of BAS 440 00 I on brood development of honeybees (*Apis mellifera* L.)
Study Author(s):	Alscher A., Claßen C., and Staffel J.
Testing Laboratory:	RIFCON GmbH, Goldbeckstraße 13, 69493 Hirschberg, Germany
Laboratory Report No.:	1440041
Sponsor Study No.:	BASF Reg. Doc. #: 2015/1001368; 742102
Study Completion Date:	14 December 2015
Data Access:	Data submitter is data owner
Data Protection Claimed:	No claim of confidentiality

II. MATERIALS AND METHODS

Test Guideline: OECD Guidance Doc. No. 75 (2007)

Deviations from Guideline:

- The study methodology for the collection of pollen samples and nectar in honey bee stomachs for the analysis of afidopyropen residues did not provide for the collection of replicate samples within the single 'residue' tunnel (tunnels used to monitor residues for afidopyropen and control tunnels were separate from those used to assess effects); instead only a single pooled sample was taken from the control and the test item-treated tunnel, respectively.
- The post-application pollen trap sample for the afidopyropen residue tunnel collected 1 DAT was supplemented with pollen collected directly from forager bees, and also from pollen

collected inside the tunnel's hive 3 DAT; therefore, this sample really represents a combined sample for 1-3 DATs.

- The reference toxicant dimethoate - which was being used in a "parallel study" - was mistakenly applied to afidopyropen treatment replicate T4, and the replicate tunnel was discarded from the study; therefore, there were only three replicates in the test item treatment group.
- The reference item (fenoxycarb) was applied at 300 g a.i./ha in this study, an application rate that is twice the recommended rate given in OECD Guidance Document No. 75 (150 g a.i./ha).
- The quantities of material applied in both the test item (afidopyropen) and the reference item (fenoxycarb) treatments was not verified analytically.
- The acclimation period for honey bee colonies in this study (7 days) is longer than what is recommended (3 days) in OECD Guidance Document No. 75; the study author stated the acclimation period was extended (and applications delayed) due to substantial precipitation that occurred -3 (7 mm), -2 (11 mm), and -1 (3 mm) days before applications were made.
- For the last 4 days of the exposure phase (4-7 DAT), and first 3 d of the monitoring period (8-10 DAT) the maximum daily temperature (32.0-39.5 °C) exceeded the recommended maximum daily temperature in the OECD guidance document (30.0 °C).

GLP Compliance: Yes; signed GLP certificate was included and reported no guideline deviations. Laboratory certified by the LUBW Landesanstalt für Umwelt, Messungen und Naturschutz Baden-Württemberg, Karlsruhe.

A. MATERIALS

Test Material: BAS 440 00 I (TEP, VERSYS™)
Test Material Identity Batch No. FD-130925-0022; a yellow, liquid formulation comprising 9.82 g/L (measured) afidopyropen (BAS 440 I: 100 g/L [nominal]).
Details on Preparation and Application of Test Materials:
The application was carried out during bee flight at full flowering of the crop (*P. tanacetifolia*) on 25 June 2015. All substances were applied in 400 L/ha water using a calibrated, portable boom sprayer (200 cm wide, 50 cm between nozzles [flat fan type, no. 5]).
Analytical Monitoring: None reported
Details on Analytical Monitoring: N/A
Reference material: Insegar™ (fenoxycarb: 300 g a.i./ha (nominal)
Reference Material Identity Batch™ SMO2K434; water-soluble granules comprising fenoxycarb: 25% w/w or 250 g/kg (nominal)
Vehicle: None

Test Organism (Species): *Apis mellifera* L. (honeybee)
Animal Group: Arthropoda/Insecta/Hymenoptera/Apidae
Details on Test Organisms: Healthy honeybee colonies, containing ten combs consisting of three to five brood combs including all brood stages and sufficient food supply,

were used for the study. The colonies for nectar/pollen sampling had two bodies, each with ten combs, instead of one body with ten combs. At the first brood assessment (DAT -1/BFD 0) colonies contained 21,800 to 31,600 brood cells with all stages present, 13,600 to 23,400 food cells, and approximately 7,150 to 10,205 bees. Bees in the colonies were free of clear visual symptoms of disease or pests (including *Varroa* mites), and no unusual occurrences were reported in colonies prior to applications. Sister queens from 2014 were used to produce colonies which are as uniform as possible (source: RIFCON GmbH).

B. STUDY DESIGN AND METHODS

Study Type: Semi-field (tunnel) study

Test Duration Type: Long-term (26 d) toxicity test

Limit Test: None reported

Total Exposure Duration: 8 d (0-7 DAT)

Post-Exposure Observation Period:

20 d (8-27DAT)

Remarks: Bee mortality was assessed daily beginning five days before, and ending 27 DAT; mortality on the day of applications was assessed shortly before and 2 h after treatment, and in the evening of the treatment day t. Mortality in the tunnels was evaluated daily using linen sheets (area approximately 18 m^2) laid at ground level in the front, middle and back of the tunnels, as well with dead zone dead bee traps at hive entrances; subsequent to colonies being removed from the tunnels, mortality at the monitoring site was evaluated using only dead zone dead bee traps. Foraging activity of the bees, and overall behavior, were assessed daily beginning 5 days before, and until 7 days after treatment while colonies were confined to tunnels. Condition of the colonies (comb area containing pollen and nectar, brood status, presence of healthy queens, and overall colony strength) and bee brood development were assessed -1, 4, 8, 15, 22 and 27 DAT (Brood Fixation Day 0; BFD0 = -1 DAT). Brood development was evaluated using digital images taken of marked cells (212-346 cells) on 1-2 brood combs in each colony using HiveAnalyzer© software. Afidopyropen residues in flowers and leaves were assessed using samples collected from all afidopyropen and control tunnels before and after (<4 h) treatments; residues in pollen (-1, 1 and 3 DAT) and in nectar from the honey stomach of forager bees (-1 and 1 DAT) were assessed using samples collected from two additional 'residue-only' tunnels.

Test Environmental Conditions:

Ambient environmental conditions inside the tunnels - weather data for -5 to 7 DAT acquired inside an adjacent tunnel not used for the study, data for 8 to 27 DAT acquired at the monitoring site - and reported here

	as ranges of daily means: 13.3-16.7 °C and 76-89% relative humidity (RH) before application; 19.2 °C and 71% RH the day of applications; 20.3-29.7 °C and 53-79% RH during 8-d exposure phase in the tunnels; 15.5-29.9 °C and 37-78% RH during 20-d phase at monitoring site. Note that 4-10 DAT the maximum daily recorded temperature was 32.0-39.5 °C. Rainfall reported on -4, -3, -2, -1, 2, 3, 11, 13, 14, 19, 23, 24, 25, and 26 DAT consisted of 1.0, 7.0, 11.0, 3.0, 10.0, 5.0, 0.5, 4.0, 1.0, 1.0, 11.0, 1.5, 13.0 and 1.0 mm, respectively.
Photoperiod and Lighting:	Natural
Nominal Concentrations:	Negative control: tap water (400 L/ha) BAS 440 00 I: 0.5 L/ha (50 g a.i./ha (nominal)) Insegar™: 1,200 g/ha (300 g a.i./ha (nominal))
Test Plots:	The test site was located in 68526 Ladenburg, Baden-Württemberg, Germany. Separate tunnels were used for the three test groups, with replicates (4x) within each test group. Tunnels (18 m length x 6 m width x 2.9 m height [108 m^2 floor space]) were set up within *ca.* 84 m^2 plots of *P. tanacetifolia*. Bees were provided with water via bird baths placed beside each hive.
Test Design:	Tunnel test under semi-field conditions; study was carried out using four tunnels (*i.e*. replicates) for each treatment group, with one bee hive per tunnel. Tunnels were set up on a field of *P. tanacetifolia*, and healthy bee colonies were introduced the evening of 25 June 2015, shortly before full flowering of the crop, and seven days before application (-7DAT). Applications were carried out during bee flight at full flowering of the crop. Bees were exposed to tap water (negative control), afidopyropen, or reference (fenoxycarb) item-treated phacelia in the tunnels for eight days. At 7 DAT, colonies were removed from the tunnels and relocated to a monitoring site approximately 5.75 km west. The monitoring site (near Hirschberg, Baden-Württemberg, Germany) was located in a forested area with no bee-attractive crops. Assessments of the persistence of afidopyropen residues in *P. tanacetifolia* flowers, leaves, pollen (pollen traps and directly from bees), and in nectar from the honey stomach of foraging bees were carried out in separate residue-monitoring tunnels simultaneous to tests for effects on honey bee brood development. Residues in leaves were also measured in afidopyropen-treated tunnels used for measuring effects. Residues in whole flowers and leaves were assessed using samples collected from test item and control tunnels before applications (sampling split between -6 and -1 DAT), and after applications (<4 h). A composite sample (≥5 g each) of flower blossoms

and leaf tissues were randomly collected from each of the test item and control tunnels (4 x), and stored at ≤-18 °C within 6 h of collection. Pollen samples (≥1 g) were collected before (-1 DAT) and after (1-3 DAT) applications in additional residue tunnels, with one tunnel for the negative control treatment, and another for the afidopyropen treatment, using a pollen trap attached to the hive in each tunnel. Foraging bees (approx. 300 bees/tunnel) for honey stomach analysis were collected -1 and 1 DAT inside the residue tunnels using a modified hand-held vacuum. Collected bees were frozen until dissection, when they were defrosted so that stomachs could be removed; collected honey stomachs were then stored at ≤-18 °C. All collected samples were shipped on dry ice to SGS Institut Fresenius GmbH (Taunusstein, Germany) for residue analysis.

III. APPLICANT'S REPORTED RESULTS AND DISCUSSION

Exposure Duration:	8 d
Endpoint(s):	No observed adverse effect level (NOAEL)
Effect Concentration:	≥ 0.5 L/ha
Basis for Concentration:	Nominal
Effect Concentration Type:	Test material
Basis for Effect:	Survival of adult bees, foraging activity, behavior, colony development, colony strength, bee brood.

Applicant-Provided Results:

Applications were made 25 June 2015 using two identically-equipped hand-held boom sprayers (one for the control and reference item, the other for the test item) between 11:49 AM and 12:39 PM (applications reportedly took 1-2 minutes/tunnel). Mean bee foraging activity prior to applications was reported to be 10.1 ± 7.0[3], 11.5 ± 6.3, and 12.2 ± 5.5 bees/m^2 in control, afidopyropen, and fenoxycarb tunnels, respectively. Wind speed outside tunnels was 0.0-0.5 m/s, cloudiness was 50%, and temperature and relative humidity inside tunnels was 22.2-24.7 °C and 39-53% RH, respectively. The amount of applied product - based on determination of the applied spray volume using a calibrated flow meter - deviated from the target application amount by -1.6 to +1.9% for afidopyropen applications, and -0.6 to 0.0% for fenoxycarb applications. Note that BFD 0 was 24 June 2015, and 0 DAT was 25 June 2015.

Sublethal Behavioral Effects: According to the study authors, a few abnormal sublethal behavioral effects were observed in afidopyropen-treated tunnels, and included coordination problems in "up to 60 worker bees per colony," and a single worker bee that was observed to have fallen from a flower on 0 and 1 DAT.

[3] Note that, unless otherwise indicated, all means reported by the study author are followed by ± one standard deviation (STD DEV).

Adult & Juvenile Mortality: According to the study author, there were no significant differences in adult bee mortality between afidopyropen, fenoxycarb and negative control groups at any time during the study. Overall numbers of dead adult bees in the pre-application period averaged 71.9 ± 24.8, 75.1 ± 27.5, and 73.3 ± 32.5 in the control, afidopyropen, and fenoxycarb groups, respectively, based on linen sheets and dead bee traps (**Table 1**). During the exposure phase of the study, overall adult mortality averaged 41.8 ± 16.2, 41.1 ± 17.3 and 60.1 ± 38.4 in the control, afidopyropen and fenoxycarb groups, respectively, based on linen sheets and dead bee traps. In the post-exposure (monitoring) phase of the study adult mortality averaged 6.2 ± 4.3, 4.5 ± 4.5, and 6.9 ± 7.0, in the control, afidopyropen and fenoxycarb groups, respectively, based on dead zone dead bee traps alone.

Pupal mortality (based on dead zone dead bee traps alone) during the pre-application phase averaged 0.2 ± 0.4, 0.1 ± 0.2 and 0.6 ± 1.4 in the control, afidopyropen and fenoxycarb tunnels, respectively (**Table 1**). During the exposure phase pupal mortality averaged 0.3 ± 0.8, 0.3 ± 1.0 and 0.4 ± 0.8 in the control, afidopyropen and fenoxycarb-treated tunnels, respectively. During the post-exposure monitoring phase, pupal mortality averaged 0.1 ± 0.3, 0.2 ± 0.6, and 2.5 ± 5.1 in the control, afidopyropen and fenoxycarb-treated colonies. The study author did not conduct statistical analyses on juvenile bee mortality data due to a very low number of dead pupae in control and test item groups.

Table 1. Study author-reported effects on honey bee (*Apis mellifera*) foraging activity, mortality (juvenile & adult worker), and brood development under semi-field conditions (tunnel test) at pre-application, in-tunnel exposure phase, and post-exposure monitoring phase for control, BAS 440 00 I (formulated afidopyropen)-treated, and fenoxycarb (reference)-treated colonies; means ± standard deviation (SD) are reported.

	Control	BAS 440 00 I	Reference Item
Mean foraging activity [n bees/m²/colony/day] during:			
Pre-application phase	10.1 ± 7.0	11.5 ± 6.3	12.2 ± 5.5
Exposure phase in the tunnels	19.6 ± 9.1	**14.4 ± 6.6†**	**18.5 ± 8.8†**
Mean mortality of worker bees [n dead bees/colony/day] during:			
Pre-application phase [1]	71.9 ± 24.8	75.1 ± 27.5	73.3 ± 32.5
Exposure phase in the tunnels [1]	36.6 ± 17.3	36.0 ± 17.8	52.6 ± 37.9
Monitoring phase outside the tunnels [2]	6.2 ± 4.3	4.5 ± 4.5	6.9 ± 7.0
Overall after application	14.9 ± 16.9	13.5 ± 17.5	19.9 ± 29.4
Mean mortality of pupae [n dead pupae/colony/day] during:			
Pre-application phase [1]	0.2 ± 0.4	0.1 ± 0.2	0.6 ± 1.4
Exposure phase in the tunnels [1]	0.3 ± 0.8	0.3 ± 1.0	0.4 ± 0.8
Monitoring phase outside the tunnels [2]	0.1 ± 0.3	0.2 ± 0.6	2.5 ± 5.1
Overall after application	0.2 ± 0.5	0.2 ± 0.7	1.9 ± 4.4
Mean brood indices at BFD 23 (22 DAT):			
Brood termination rate at BFD 23 [%]	27.9 ± 13.1	23.6 ± 14.9	66.7 ± 28.2

Brood-index at BFD 23	3.6 ± 0.7	3.8 ± 0.7	1.7 ± 1.4
Brood compensation-index at BFD 23	4.1 ± 0.5	4.3 ± 0.4	**3.1 ± 0.9***

[1)] Sum of dead individuals found in dead bee traps and on linen sheets in the tunnels.
[2)] Mean number of dead honeybees per day and colony found in dead bee traps.
* = statistically significant differences ($p < 0.05$) compared to the control, Dunnett's test
† = statistically significant differences ($p < 0.05$) compared to the control, Mann-Whitney test
DAT = days after treatment

Colony Strength: There were no significant differences across treatment groups in the mean number of bees per colony at any point in the study (see **Table 2**). In terms of brood (cells with eggs, larvae plus pupae), at -1 DAA there were an average of 25,950 ± 3181, 28,067 ± 3101, and 26,750 ± 3722 brood for control, afidopyropen and fenoxycarb colonies, respectively. At the end of the exposure phase, there were 19,800 ± 6154, 22,200 ± 1908, and 17,400 ± 2257 brood/colony in the control, afidopyropen, and reference colonies, respectively. At the end of the monitoring phase, there were 23,950 ± 3126, 25,400 ± 2078, and 22,650 ± 681 brood/colony in the negative control, afidopyropen, and fenoxycarb colonies, respectively (**Table 3**). According to the study authors, the mean number of cells containing brood in the fenoxycarb colonies decreased from the second assessment and remained less than in negative controls and afidopyropen colonies for the remainder of the study; this reduction was considered evidence that the study design was responsive (sensitive to) the reference chemical (fenoxycarb). The number of cells containing pupae at 8 DAT had declined by 9, 5 and 32% in the control, afidopyropen and fenoxycarb treatments, and decreased by 39%, 28% and 71% in these respective groups by 15 DAT (Table 4).

Foraging Activity: The study author reported that due to inclement weather there was very little foraging activity on -2 and -3 DAT, and so these assessment days were excluded from associated statistical analyses. Foraging activity over the entire exposure phase of the study averaged 19.6 ± 9.1, 14.4 ± 6.6 and 18.5 ± 8.8 bees/m^2/colony/d in the control, afidopyropen and fenoxycarb-treated tunnels, respectively (**Table 1**). According to the study author, mean foraging activity was significantly ($p < 0.05$) different (*i.e.*, lower) in the afidopyropen-treated tunnels compared to negative control tunnels immediately following applications (0 DAT), and during the exposure period. Foraging activity during the exposure period was also significantly ($p < 0.05$) different (*i.e.*, lower) in fenoxycarb-treated tunnels compared to the negative control tunnels during the exposure phase.

Condition of the Colonies: According to the study authors, the evaluation of brood at -1 DAT indicated healthy colonies with queens and all brood stages present, and a sufficient supply of nectar and pollen. Following treatment applications, no differences were reported in the quantity of brood or food in afidopyropen- or fenoxycarb-treated colonies relative to negative control colonies; no additional feeding was provided during the exposure and monitoring phases of the study.

Table 2. Mean colony size (bees/colony) relative to (as percentage of, %) one day before application (-1 DAT, BFD0) mean colony size (mean ± SD) in negative control, formulated afidopyropen (BAS 440 00 I), and fenoxycarb (reference) treatment groups, as reported by the study author.

Treatment	-1 DAT	4 DAT	8 DAT	15 DAT	22 DAT	27 DAT

Control	8564 ± 1342	8596 ± 1291 (0)	10904 ± 2160 (+27)	13536 ± 3193 (+58)	10699 ± 3567 (+28)	10936 ± 3042 (+28)
BAS 440 00 I	8190 ± 362	7908 ± 823 (-3)	9880 ± 469 (+21)	12610 ± 1298 (+54)	12935 ± 874 (+58)	12697 ± 521 (+55)
Reference Item	8873 ± 1042	8255 ± 159 (-7)	10303 ± 312 (+16)	12951 ± 973 (+46)	9214 ± 496 (+4)	9344 ± 674 (+5)

DAT = days after treatment.

Table 3. Summary of total number of brood (eggs, larvae and pupae) in control, formulated afidopyropen (test item) and fenoxycarb (reference) colonies at specified days after application (DAA). Table reproduced from BASF Study ID 742102.

Date [DD.MM. YYYY]	DAA/ BFD	Control group			Test item group			Reference item group		
		Absolute [n] [1)]		Relative develop-ment [2)]	Absolute [n] [1)]		Relative develop-ment [2)]	Absolute [n] [1)]		Relative develop-ment [2)]
		Mean	± SD		Mean	± SD		Mean	± SD	
24.06.2015	-1/0	25,950	3,181	-	28,067	3,101	-	26,750	3,722	-
29.06.2015	4/5	23,350	1,628	-10 %	25,333	2,532	-10 %	21,250	3,924	-21 %
03.07.2015	8/9	19,800	3,154	-24 %	22,200	1,908	-21 %	17,400	2,257	-35 %
10.07.2015	15/16	20,350	915	-22 %	22,133	945	-21 %	17,100	1,936	-36 %
17.07.2015	22/23	21,300	2,094	-18 %	23,400	1,908	-17 %	20,050	998	-25 %
22.07.2015	27/28	23,950	3,126	-8 %	25,400	2,078	-9 %	22,650	681	-15 %

DAA = days after application; BFD = brood area fixing day; [1)] absolute mean strength of the colonies ± standard deviation; [2)] relative development of the mean strength of the colonies (strength of the colonies at the first assessment was set as basis)

Table 4. Summary of total number of pupae in control, formulated afidopyropen (test item) and fenoxycarb (reference) colonies at specified days after application (DAA). Table reproduced from BASF Study ID 742102.

Date [DD.MM. YYYY]	DAA/ BFD	Control group			Test item group			Reference item group		
		Absolute [n] [1)]		Relative develop-ment [2)]	Absolute [n] [1)]		Relative develop-ment [2)]	Absolute [n] [1)]		Relative develop-ment [2)]
		Mean	± SD		Mean	± SD		Mean	± SD	
24.06.2015	-1/0	13,200	2,033	-	14,600	346	-	15,200	2,304	-
29.06.2015	4/5	13,950	2,144	+6 %	16,333	1,026	+12 %	15,800	4,484	+4 %
03.07.2015	8/9	11,950	1,949	-9 %	13,867	1,102	-5 %	10,400	2,971	-32 %
10.07.2015	15/16	8,100	1,352	-39 %	10,533	115	-28 %	4,450	1,684	-71 %
17.07.2015	22/23	10,800	1,751	-18 %	11,800	529	-19 %	8,250	1,652	-46 %
22.07.2015	27/28	12,900	2,914	-2 %	13,000	1,929	-11 %	10,350	252	-32 %

DAA = days after application; BFD = brood area fixing day; [1)] absolute mean number of pupae cells of the colonies ± standard deviation; [2)] relative development of the mean strength of the colonies (strength of the colonies at the first assessment was set as basis)

Brood Indices: According to the study author, there were no significant differences in the brood termination rate or brood index between the negative control and afidopyropen treatment groups at any point in the study. The mean brood termination rate and brood index for the fenoxycarb-treated colonies were significantly ($p < 0.05$) different (*i.e.*, higher and lower, respectively) relative to negative control colonies during the exposure period. The mean brood compensation index in the fenoxycarb-treated tunnels was significantly ($p < 0.05$) different (*i.e.*, lower) relative to negative control tunnels throughout the study. According to study authors, the brood compensation index for each of the treatment groups were higher than the corresponding brood index, indicating that cells with terminated brood were refilled with new eggs. The mean brood compensation index in negative control colonies were lower than in afidopyropen-treated colonies, which the authors believed indicated that that afidopyropen caused no adverse effect on bee brood development.

Residues: The study author reported that no residues of either BAS 440 I (afidopyropen) or its photolysis metabolite M4401007 were found in flower, leaf, nectar or pollen specimens collected at random locations in tunnels before applications were made. No residues of either compound were reportedly found in specimens collected in negative control and treatment tunnels following applications. Immediately (<4 h) following application, afidopyropen residues in *Phacelia* flowers and leaves were 3.03-4.67 and 0.43-2.97 mg a.i./kg, respectively; M4401007 residues in flowers and leaves were 2.37-3.46 and 0.57-3.61 mg a.i./kg, respectively. Afidopyropen residues in pollen and nectar specimens were 0.17 mg a.i./kg and <limit of quantification (LOQ=0.01 mg a.i./kg), respectively; M4401007I residues in pollen and nectar specimens were 0.06 mg a.i./kg and <LOQ (LOQ=0.01 mg a.i./kg), respectively.

Weather Data: Weather data reported by the study author is summarized in **Figure 1**, and includes total daily precipitation (mm), daily mean temperature (°C), and daily mean relative humidity (% RH). The study author noted that cloudiness was recorded during the entire study period; rainfall was recorded -3 to -1 DAT, and rainfall exceeded 10 mm on -2, 2, 23, and 25 DAT. Temperatures 6 to 10 DAT were reportedly very high (mean up to 29.9 °C, maximum up to 39.5 °C), and the study author speculated this could have affected brood development and/or bee behavior.

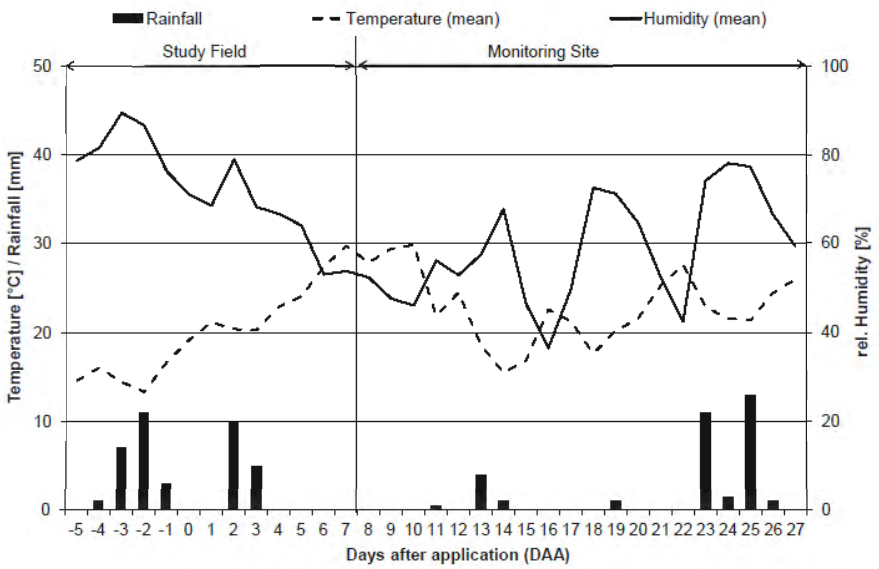

Figure 1. Weather data at study and monitoring sites, as reported by study author.

The study author concluded that overall, formulated afidopyropen (BAS 440 00 I) applied at a nominal rate of 50 g a.i./ha while bees were actively foraging did not adversely affect honeybee colonies in this study, despite short-term effects on adult mortality, behavior and transient reduction in foraging activity.

Applicant-Reported Statistics and Error Estimates

The applicant reported means and standard deviations for all endpoints, included applicant-calculated brood development indices. Mortality and bee brood development index data were tested for normality (Shapiro-Wilk's test) and homogeneity of variances (Bartlett's test) prior to analyses. Parametric ANOVA and Dunnett's tests were used for data that were approximately normally distributed and homoscedastic, and Kruskal-Wallis analyses and Mann-Whitney pairwise tests used if data were not, with $\alpha = 0.05$. Two-sided tests were used for pre-exposure data, and one-sided tests were used on the remaining data.

IV. OVERALL REMARKS, ATTACHMENTS

The registrant submitted Microsoft Excel spreadsheets containing most of the study author's biological evaluation data, brood development index calculations, along with an OECD-formatted summary.

V. PRIMARY REVIEWER'S ANALYSIS AND CONCLUSIONS

Reviewer results for afidopyropen effects on adult foraging activity, adult (worker) bee and juvenile mortality, and honey bee brood development are summarized in **Table 5**.

Foraging Activity: Mean foraging activity was significantly ($p < 0.05$) different (*i.e.*, 31% lower) in afidopyropen-treated (14.64 bees/m^2/colony/d)[4] tunnels compared to negative control tunnels (21.21 bees/m^2/colony/d) during the exposure period (0-8 DAT).

Adult & Juvenile Mortality: Mean adult honey bee mortality was significantly ($p < 0.05$) different (*i.e.*, 29% lower) in afidopyropen-treated tunnels compared to negative control tunnels during the exposure (BAS 440 I: 23.22 dead bees/colony/d; control: 32.50 dead bees/colony/d) and the monitoring period (BAS 440 I: 4.47 dead bees/colony/d; control: 6.20 dead bees/colony/d) (28% lower).

Mean juvenile (specifically pupae) mortality was significantly ($p < 0.05$) different (*i.e.*, higher) in afidopyropen- and fenoxycarb-treated tunnels compared to negative control tunnels during the monitoring period ((BAS 440 I: 0.23 dead pupae/colony/d (2.9-fold higher); fenoxycarb: 2.43 dead pupae/colony/d (30-fold higher); control: 0.08 dead pupae/colony/d).

Table 5. Reviewer-calculated effects on honey bee (*Apis mellifera*) foraging activity, mortality (juvenile & adult worker), and brood development under semi-field conditions (tunnel test) at pre-application, in-tunnel exposure phase, and post-exposure monitoring phase for control, formulated

[4] Note that, unless otherwise indicated, all means reported by the reviewer are followed by ± one standard error (SE).

afidopyropen (BAS 440 00 I)-treated, and fenoxycarb (reference)-treated colonies; means ± standard error (SE) are reported.

	Control	BAS 440 00 I	Reference Item
Mean foraging activity [n bees/m²/colony/day] during:			
Pre-application phase	6.94 ± 1.43	7.65 ± 1.75	8.40 ± 1.43
Exposure phase in the tunnels	21.21 ± 1.06	**14.64 ± 0.88***	19.35 ± 1.02
Mean mortality of adult worker bees [n dead bees/colony/day] during:			
Pre-application phase [1]	71.83 ± 5.07	57.17 ± 10.43	73.21 ± 6.63
Exposure phase in the tunnels [1]	32.50 ± 2.69	**23.22 ± 4.15†**	45.89 ± 6.49
Monitoring phase outside the tunnels [2]	6.20 ± 0.49	**4.47 ± 0.59†**	6.83 ± 0.78
Overall after application	14.36 ± 1.44	**10.29 ± 1.63†**	18.95 ± 2.67
Mean mortality of pupae [n dead pupae/colony/day] during:			
Pre-application phase [1]	0.17 ± 0.08	0.06 ± 0.06	*Not reported*
Exposure phase in the tunnels [1]	0.39 ± 0.14	0.22 ± 0.19	*Not reported*
Monitoring phase outside the tunnels [2]	0.08 ± 0.03	**0.23 ± 0.08†**	**2.43 ± 0.57†**
Overall after application	0.17 ± 0.05	0.23 ± 0.08	**2.43 ± 0.57† [3]**

[1)] Sum of dead individuals found in dead bee traps and on linen sheets in the tunnels.
[2)] Mean number of dead honeybees per day and colony found in dead bee traps.
[3)] 'Overall after application' value for the reference item treatment group only includes data from the monitoring period of the study.
* = statistically significant differences ($p < 0.05$) compared to the control, Dunnett's test
† = statistically significant differences ($p < 0.05$) compared to the control, Wilcoxon Rank Sum test

Strength and Condition of the Colonies: There were no statistically significant differences in the overall quantity of brood in afidopyropen- or fenoxycarb-treated colonies relative to negative control colonies at any time during the study; however, across treatments, the number of brood cells was significantly ($p <0.05$) different (*i.e.*, lower) at BFDs 5 and 9 (*i.e.*, during the exposure period) relative to BFD 0 (see **Appendix I**). There were no significant differences in the overall quantity of food cells in test or reference item-treated colonies relative to control colonies, or at any time in the study relative to BFD 0.

There were no significant differences between treatment groups in the mean number of adult bees per colony at any point in the study (see **Table 6**). Mean colony strength at 27 DAT (BFD28) for the negative control, afidopyropen- and fenoxycarb-treated colonies was 10,936 ± 1521, 12,697 ± 301, and 9,344 ± 337 adult bees, respectively. The mean number of pupae was significantly ($p <0.05$) different (*i.e.*, 24% lower) in fenoxycarb-treated colonies during the monitoring phase of the study (mean = 8,363 ± 756 pupae/colony) relative to negative control colonies (10,938 ± 656 pupae/colony).

Table 6. Reviewer-calculated mean colony size relative to (as percentage of) one day before application (-1 DAT, BFD0) mean colony size (mean ± SE) in negative control, formulated afidopyropen (BAS 440 00 I) and fenoxycarb (reference) colonies.

	-1 DAT (n)	4 DAT (%)	8 DAT (%)	15 DAT (%)	22 DAT (%)	27 DAT (%)
Control	8564 ± 671	0.8 ± 5.9	+28.5 ± 13.6	+59.6 ± 20.3	+28.8 ± 21.7	+28.1 ± 17.5

BAS 440 00 I	8190 ± 209	-3.5 ± 4.3	+20.7 ± 3.3	+53.8 ± 6.7	+58.1 ± 7.1	+60.8 ± 6.9
Reference Item	8873 ± 521	-5.8 ± 6.8	+17.8 ± 9.4	+48.2 ± 13.4	+4.1 ± 7.1	+5.9 ± 3.8

DAT = days after treatment.

Brood indices: The brood index (bi) is used as an indicator of bee brood development, where cells are classified from 0 to 5 (0 = empty; 1 = egg; 2 = young larvae; 3 = old larvae; 4 = capped brood; 5 = empty after hatching or filled again with new brood) based on whether a particular cell has reached its expected brood development stage on each observation day. At BFD 5 (4 DAT), BFD 16 (15 DAT) and BFD 23 (22 DAT) the mean brood index for fenoxycarb-treated colonies was significantly ($p < 0.05$) different (*i.e.*, lower) compared to negative control colonies (**Table 7**).

The brood compensation index is similar to the brood index, but quantifies colony recovery rather than whether cells are at their expected brood stage, and uses the same 0 to 5 classification scheme. At BFD 5 (4 DAT) the mean brood compensation index for fenoxycarb-treated colonies was significantly ($p < 0.05$) different (*i.e.*, lower) compared to control colonies (**Table 7**).

The brood termination rate is the percentage of brood cells that do not successfully transition from egg to hatched worker bees. In this study there were no statistically significant differences in either the afidopyropen- or fenoxycarb-treated colonies compared to negative control colonies at any assessment date; however, overall mean brood termination rates (and variability measured as standard error) for fenoxycarb-treated colonies were higher than in afidopyropen-treated or negative control colonies (**Table 7)**.

Table 7. Reviewer-calculated honey bee (*Apis mellifera*) brood development metrics - brood index (bi), brood compensation index (bci), and brood termination rate (%, btr) - under semi-field conditions (tunnel test) during in-tunnel exposure phase (BFD 5), and post-exposure monitoring phase (BFDs 9, 16 and 23) for negative control, formulated afidopyropen (BAS 440 00 I)-treated, and fenoxycarb (reference)-treated colonies; means ± standard error (SE) are reported.

	BFD 5 (4 DAT)	BFD 9 (8 DAT)	BFD 16 (15 DAT)	BFD 23 (22 DAT)
Brood Index (bi)				
Control	2.23 ± 0.57	2.99 ± 0.87	2.90 ± 0.89	3.62 ± 1.12
BAS 440 00 I	2.33 ± 0.60	3.34 ± 0.86	3.06 ± 0.98	3.82 ± 1.23
Reference Item	**1.00 ± 0.58***	1.67 ± 0.99	**1.47 ± 0.97***	**1.84 ± 1.21***
Brood Compensation Index (bci)				
Control	2.27 ± 0.55	3.07 ± 0.83	3.11 ± 0.81	4.12 ± 0.79
BAS 440 00 I	2.35 ± 0.58	3.41 ± 0.81	3.18 ± 0.97	4.27 ± 0.79
Reference Item	**1.07 ± 0.56***	1.81 ± 0.96	1.98 ± 0.98	3.16 ± 0.85
Brood Termination Rate (btr)				
Control	19.13 ± 5.65	25.62 ± 6.37	27.85 ± 6.60	28.09 ± 6.68
BAS 440 00 I	14.25 ± 6.59	16.42 ± 6.87	23.58 ± 7.32	23.69 ± 7.36
Reference Item	59.62 ± 15.44	62.50 ± 15.29	66.87 ± 13.99	66.87 ± 13.99

* = statistically significant differences ($p < 0.05$) compared to the control, Dunnett's test
BFD = brood fixing day
DAT = days after treatment

Residues: A single pooled sample was collected from all (4 tunnels/treatment) afidopyropen and negative control tunnels for analysis of afidopyropen residues in flowers and leaves, allowing for statistical analysis of treatment means; samples for analysis of residues in pollen and nectar were collected from a single residue-only tunnel, so no analyses could be carried out on reported residue results for nectar and pollen residues.

Residues of parent afidopyropen (BAS 440 I) and its dimer (M4401007) were below the analytical level of detection (LOD = 0.003 mg a.i./kg) in leaves and flowers collected both before and after applications in all control treatment tunnels. Similarly, residues of both compounds were below the LOD in afidopyropen-treated tunnels prior to applications. Immediately (<4 h) following applications afidopyropen residues in *Phacelia* flowers and leaves were 2.32 ± 0.78 and 1.68 ± 0.53 mg a.i./kg, respectively; M4401007 residues in flowers and leaves were 1.59 ± 0.51 and 2.00 ± 0.54 mg a.i./kg, respectively. Afidopyropen residues in pollen and nectar specimens were 0.17 mg/kg and <LOQ (0.01 mg/kg), respectively; the dimer M4401007I residues in pollen and nectar specimens were 0.06 mg/kg and <LOQ, respectively.

Reviewer's Statistical Verification:
The applicant's calculations (including brood development indices) were verified by the reviewer, and statistical analyses confirmed using R (ver. 3.2.5)[5] statistical software, and the multcomp[6] analysis package. The reviewer relied on the Shapiro-Wilk's test and Bartlett's test to evaluate whether data were normally distributed or homoscedastic, respectively. Parametric ANOVA and Dunnett's test were used to test for statistical differences amongst means for data that met assumptions for parametric tests (*i.e.,* data were approximately normally distributed and had homogenous variances), and Kruskal-Wallis and Wilcoxon Rank Sum test were used for non-parametric comparisons. One-sided tests were used for all hypothesis-based testing; $\alpha = 0.05$ for all mean comparison tests, and $\alpha = 0.01$ for all assumptions testing.

See **Appendix I** for summary statistics and diagnostic tests (*i.e.,* goodness of fit and equivalent variances tests) for all data described in this data evaluation report.

Based on statistically significant adverse effects in the afidopyropen-treated colonies, the no-observed adverse effect level (NOAEL) across the various measurement endpoints for adult honey bees and developing brood is <50 g a.i./ha under the conditions tested.

Reviewer's Comments:

[5] R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/.
[6] Hothorn T, F Bretz and P Westfall. 2008. Simultaneous inference in general parametric models. Biometric Journal 50: 346-363.

Data provided in the study report indicated that the average time to make applications to each tunnel was 1-2 minutes per tunnel. Given the described application protocols in the study report it's difficult to understand how applications could have been made to each of the 12 tunnels in such a short timeframe.

The fourth test item colony/tunnel ("T4" in the study report) was reported to have been accidentally treated with dimethoate (which was being used in a "parallel study" according to the study author) at the time of initial applications; therefore, this replicate was excluded from all biological evaluations by both the study author and the reviewer. The study author also reported that due to inclement weather there was very little foraging activity on -2 and -3 DAT, and so these assessment days were excluded from associated statistical analyses.

The study author reported that not enough pollen could be collected from pollen traps 1 DAT in the afidopyropen-treated residue tunnel, and so the pooled sample was supplemented with pollen taken from the pollen loads of forager bees, and additionally with comb pollen collected 3 DAT from inside hives. The addition of pollen collected from forager bees, and pollen from inside the hive collected several days later, to the pooled sample potentially allows for the introduction of non-*Phacelia* pollen, or the introduction of pollen that was not treated with the test item, and in both cases this could serve to dilute afidopyropen residues.

For a seven-day period spanning the end of the in-tunnel exposure phase and the beginning of the remote monitoring phase, the maximum daily recorded temperature was 32.0-39.5 °C. OECD Guidance Document No. 75 notes that daytime temperatures exceeding 30 °C may stop nectar secretion.

Additionally, rainfall exceeding 10 mm was reported several times during the study (-2, 2, 23, and 25 DAT), and rainfall -3, -1 and 13 DAT exceeded 3 mm. Excessive precipitation was implicated by the study author in severely reduced honey bee foraging activity -3 and -2 DAT (leading to the study author excluding data from these days from their analyses), and may well have influenced adult bee mortality and brood development; effects on early brood development could not be evaluated this early in the study because the first brood development assessment day was not until 4 DAT (BFD 5).

During the pre-application phase of the study there was high adult mortality in all study tunnels, with mean adult mortality of 71.83 ± 5.07, 57.17 ± 10.43, and 73.21 ± 6.63 dead bees/colony/d in control, afidopyropen, and fenoxycarb-treated colonies, respectively. Overall adult mortality (pooled across treatments) during the pre-application phase of the study (68.33 ± 4.18 dead bees/colony/d) was significantly ($p < 0.05$) different (*i.e.,* higher) relative to adult mortality during the exposure (34.84 ± 2.92 dead bees/colony/d) or monitoring (5.95 ± 0.38 dead bees/colony/d) phases of the study. Additionally, foraging activity during the pre-application phase of the study (mean = 7.67 ± 0.87 bees/m^2) was significantly lower ($p < 0.05$) than during the exposure phase of the study (mean = 18.74 ± 0.62 bees/m^2). High adult honey bee mortality during the pre-application phase of the study increases uncertainty in regards to the reliability of the study results.

Study results indicate that the reference item (fenoxycarb) did not result in significantly different adult honey bee mortality or brood development impacts during the exposure phase of the study relative to the negative control treatment, but did result in significantly ($p < 0.05$) different (*i.e.,* higher) pupal mortality during the monitoring phase of the study. The lack of significant effects by fenoxycarb on adult mortality or brood development - especially given the high application rate - impart additional uncertainty as to whether honeybee colonies in this study were correctly exposed to test materials, and whether the test system was able to detect treatment effects associated with the reference toxicant or afidopyropen.

The reviewer included foraging activity records from -2 and -3 DAT in analyses, which were excluded by the study author due to reports of inclement weather on these days. Inclement weather probably affected honey bee foraging activity, but foraging activity data from -2 and -3 DAT reflect real conditions encountered during the study. Including data from these days in analyses lowered the mean foraging activity value for the pre-application period for negative control, afidopyropen, and fenoxycarb-treated tunnels roughly equally (*i.e*., by 31, 33, and 31%, respectively).

The study author's calculations of mean colony strength involved taking the arithmetic sum of both sides (2) of 10 frames in a given colony, and a 'body' value. This 'body' value was included on the raw data tables appended to the study report, but were not included in the raw data spreadsheets submitted by the registrant. The reviewer accounted for this omission and added the missing 'body' value to the submitted spreadsheets, and included this value in mean colony strength calculations.

Reviewer's Conclusions:
The semi-field (tunnel) bee brood study was conducted in June-July 2015 with the formulated end-use product BAS 440 00 I (TEP, VERSYS™, 9.7% afidopyropen). Bee colonies in the negative control, reference item (fenoxycarb: 300 g a.i./ha nominal), and 50 g a.i./ha afidopyropen (BAS 440 00 I) treatments were assessed at multiple time points and photographic records were maintained; treatment rates were not confirmed analytically. The acclimation period prior to applications was seven days, the exposure period was seven days (0-7 DAT), and the post-exposure monitoring period was 26 days (8-27 DAT).

In summary, afidopyropen treatments resulted in significantly ($p < 0.05$) different (*i.e.*, 31% lower) foraging activity during the exposure period (14.64 bees/m^2) of the study relative to the control (21.21 bees/m^2). Afidopyropen treatments also resulted in significantly ($p < 0.05$) different (*i.e.*, lower) adult worker bee mortality during the exposure (23.22 dead bees/colony/day; 29% lower) and monitoring periods (4.47 dead bees/colony/day; 28% lower) of the study, relative to the negative control treatments (32.50 dead bees/colony/day, and 6.20 dead bees/colony/day, respectively). The mean mortality of pupae was significantly ($p < 0.05$) different (*i.e.*, higher) in afidopyropen- and fenoxycarb-treated tunnels compared to negative control tunnels during the monitoring period (BAS 440 I: 0.23 dead pupae/colony/d (2.9-fold higher); fenoxycarb: 2.43 dead pupae/colony/d (30-fold higher); control: 0.08 dead pupae/colony/d). There were no treatment-related differences in brood or food quantity at any time point in the study. There were no statistically significant differences in brood development

indices for afidopyropen-treated colonies relative to the negative control, but during the exposure and monitoring periods the mean brood index and brood compensation index for fenoxycarb-treated colonies were significantly lower than in negative control colonies. While no sublethal behavioral effects were reported in control tunnels, afidopyropen treatments resulted in sublethal behavioral effects (*i.e.*, "coordination problems") for roughly 60 forager bees hours after applications were made.

There were adverse weather conditions during the pre-application period (*i.e.*, rainfall -4 to -1 DAT totaled 22 mm) and spanning the end of the exposure period and the beginning of the monitoring period (*i.e.*, maximum daily temperatures > 30 °C). Increased mortality of honeybees in all treatment colonies during the pre-application period of the study may have limited the capacity of the study to detect treatment effects. In particular, colonies exhibited significantly ($p < 0.05$) different (*i.e.*, elevated) adult mortality (>68 dead bees/colony/d) during the pre-application phases of the study; these unexplained outcomes indicate that overall the study results should be interpreted with caution. Additionally, because nominal treatment levels of afidopyropen and fenoxycarb were not verified analytically, there is uncertainty regarding actual exposure levels.

The study was consistent with OECD Guidance Document 75, and indicates that honey bee colonies treated with formulated afidopyropen at 50 g a.i./ha exhibited significant adverse effects on foraging activity and pupae survival. Adult honeybee mortality was significantly lower in afidopyropen-treated colonies relative to control colonies, and so this effect is not considered adverse. Based on this study and the statistically significant effects on foraging activity and reduced pupal survival, the NOAEL is <50 g a.i./ha.

EPA Classification: Supplemental (should only be used qualitatively)
PMRA Classification: Reliable with restrictions

APPENDIX I. Output of Statistics Verified by the Reviewer

A. Summary Statistics & Tests

Honeybee Foraging Activity (bees/m²/d)

```
Call: lm(formula = value ~ group.phase + group.trtmnt, data = forage)

Residuals:
     Min       1Q   Median       3Q      Max
-20.3429  -5.5632  -0.0782   4.8752  18.9571

Coefficients:
                 Estimate Std. Error t value Pr(>|t|)
(Intercept)       20.3429     0.8729  23.306   <2e-16 ***
group.phasepre   -11.0782     1.1087  -9.992   <2e-16 ***
group.trtmntref   -0.9648     1.1580  -0.833   0.4056
group.trtmnttest  -4.5898     1.2508  -3.670   0.0003 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 7.681 on 238 degrees of freedom
Multiple R-squared:  0.3244,      Adjusted R-squared:  0.3159
F-statistic: 38.09 on 3 and 238 DF,  p-value: < 2.2e-16

Shapiro-Wilk normality test
W = 0.99228, p-value = 0.2375
```

Pre-application Phase

```
Bartlett test of homogeneity of variances
Bartlett's K-squared = 0.077999, df = 2, p-value = 0.9618

Analysis of Variance Table
Response: forage.pre$activity
                 Df Sum Sq Mean Sq F value Pr(>F)
forage.pre$trtmnt  2   25.2  12.617  0.2498 0.7798
Residuals         63 3182.5  50.517
```

Exposure Phase

```
Bartlett test of homogeneity of variances
Bartlett's K-squared = 5.8152, df = 2, p-value = 0.05461

Call:
lm(formula = forage.exp$activity ~ forage.exp$trtmnt, data = forage.exp)

Residuals:
     Min       1Q   Median       3Q      Max
-21.2141  -4.9375  -0.1906   4.9562  18.0859

Coefficients:
                     Estimate Std. Error t value Pr(>|t|)
(Intercept)           21.2141     0.9721  21.823  < 2e-16 ***
forage.exp$trtmntref  -1.8703     1.3747  -1.361    0.175
forage.exp$trtmnttest -6.5766     1.4849  -4.429 1.67e-05 ***
---
```

```
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 7.777 on 173 degrees of freedom
Multiple R-squared:  0.1047,      Adjusted R-squared:  0.09431
F-statistic: 10.11 on 2 and 173 DF,  p-value: 7.032e-05

Simultaneous Tests for General Linear Hypotheses
Multiple Comparisons of Means: Dunnett Contrasts

Fit: lm(formula = value ~ group, data = forage.exp)

Linear Hypotheses:
                 Estimate Std. Error t value Pr(>|t|)
ref - cont == 0    -1.870      1.375  -1.361    0.299
test - cont == 0   -6.577      1.485  -4.429 3.33e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Adjusted p values reported -- single-step method)
```

Adult Honeybee Mortality (no. dead bees/colony/d)

```
Call: lm(formula = value ~ group.phase + group.trtmnt, data = mort.adult)

Residuals:
    Min      1Q  Median      3Q     Max
-61.582  -7.582  -1.094   4.023 119.987

Coefficients:
                 Estimate Std. Error t value Pr(>|t|)
(Intercept)        34.977      2.469  14.165   <2e-16 ***
group.phasemon    -28.884      2.469 -11.697   <2e-16 ***
group.phasepre     33.495      3.242  10.330   <2e-16 ***
group.trtmntref     4.036      2.439   1.655   0.0988 .
group.trtmnttest   -5.890      2.634  -2.236   0.0259 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 20.4 on 380 degrees of freedom
Multiple R-squared:  0.5805,      Adjusted R-squared:  0.5761
F-statistic: 131.4 on 4 and 380 DF,  p-value: < 2.2e-16

Shapiro-Wilk normality test
W = 0.82564, p-value < 2.2e-16
```

Pre-application Phase

```
Bartlett test of homogeneity of variances
Bartlett's K-squared = 6.5111, df = 2, p-value = 0.03856

Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 1.6207, df = 2,
p-value = 0.4447
```

Exposure Phase

```
Bartlett test of homogeneity of variances
Bartlett's K-squared = 27.294, df = 2, p-value = 1.183e-06

Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 9.6833, df = 2, p-value = 0.007894

Pairwise comparisons using Wilcoxon rank sum test

     cont  ref
ref  0.404 -
test 0.020 0.016

P value adjustment method: holm
```

Monitoring Phase

```
Bartlett test of homogeneity of variances
Bartlett's K-squared = 22.059, df = 2, p-value = 1.622e-05

Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 12.082, df = 2, p-value = 0.002379

Pairwise comparisons using Wilcoxon rank sum test

     cont   ref
ref  0.6595 -
test 0.0019 0.0158

P value adjustment method: holm
```

Overall Post-application Phase

```
Bartlett test of homogeneity of variances
Bartlett's K-squared = 59.486, df = 2, p-value = 1.21e-13

Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 14.181, df = 2, p-value = 0.0008332

Pairwise comparisons using Wilcoxon rank sum test

     cont   ref
ref  0.9672 -
test 0.0013 0.0033
```

Juvenile Honeybee Mortality (no. dead pupae/colony/d)

```
Call: lm(formula = value ~ group.phase + group.trtmnt,
data = mort.juv)

Residuals:
    Min      1Q  Median      3Q     Max
-2.4250 -0.3338 -0.1592 -0.1306 20.5750

Coefficients:
                 Estimate Std. Error t value Pr(>|t|)
(Intercept)       0.30522    0.35407   0.862    0.389
```

```
group.phasemon   -0.17460    0.39037  -0.447    0.655
group.phasepre   -0.19841    0.51258  -0.387    0.699
group.trtmntref   2.29439    0.38771   5.918  8.4e-09 ***
group.trtmnttest  0.02857    0.33219   0.086    0.932
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 2.573 on 320 degrees of freedom
Multiple R-squared:  0.1257,      Adjusted R-squared:  0.1147
F-statistic:  11.5 on 4 and 320 DF,  p-value: 9.818e-09

Shapiro-Wilk normality test
W = 0.46463, p-value < 2.2e-16

Bartlett test of homogeneity of variances
Bartlett's K-squared = 611.25, df = 2,
p-value < 2.2e-16
```

Pre-application Phase

```
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 1.182, df = 1,
p-value = 0.277
```

Exposure Phase

```
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 3.0087, df = 1, p-value = 0.08282
```

Monitoring Phase

```
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 26.756, df = 2, p-value = 1.549e-06

Pairwise comparisons using Wilcoxon rank sum test

     cont    ref
ref  4.1e-06 -
test 0.047   0.005

P value adjustment method: holm
```

Overall Post-application Phase

```
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 25.19, df = 2, p-value = 3.389e-06

Pairwise comparisons using Wilcoxon rank sum test

     cont    ref
ref  2.6e-05 -
test 0.70022 0.00034

P value adjustment method: holm
```

Colony Condition - Brood (no. cells/colony/d as brood)
#full model

```
Call: lm(formula = bc.brood$n ~ bc.brood$trtmnt + bc.brood$bfd, data = bc.brood)
```

```
Residuals:
   Min     1Q Median     3Q    Max
 -4767  -1833   -400   2028   6795

Coefficients:
                    Estimate Std. Error t value Pr(>|t|)
(Intercept)          4866.55     440.65  11.044   <2e-16 ***
bc.brood$trtmntref   -217.40     481.58  -0.451    0.652
bc.brood$trtmnttest   138.74     516.19   0.269    0.789
bc.brood$bfd           33.34      20.72   1.609    0.110
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 2359 on 129 degrees of freedom
Multiple R-squared:  0.02317,     Adjusted R-squared:  0.0004531
F-statistic:  1.02 on 3 and 129 DF,  p-value: 0.3861

Shapiro-Wilk normality test
W = 0.96162, p-value = 0.000846

#trtmnt
Bartlett test of homogeneity of variances
data:  bc.brood$n by bc.brood$bfd
Bartlett's K-squared = 2.8491, df = 2, p-value = 0.2406

Kruskal-Wallis rank sum test
data:  bc.brood$n by bc.brood$trtmnt
Kruskal-Wallis chi-squared = 0.92508, df = 2, p-value = 0.6297

#bfd
Bartlett test of homogeneity of variances
Bartlett's K-squared = 10.228, df = 5, p-value = 0.06902

Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 24.159, df = 5, p-value = 0.0002024

Pairwise comparisons using Wilcoxon rank sum test
data:  bc.brood$n and bc.brood$bfd

   0      5      9      16     23
5  0.0349 -      -      -      -
9  0.0089 1.0000 -      -      -
16 1.0000 0.0222 0.0104 -      -
23 1.0000 0.1305 0.0391 1.0000 -
28 1.0000 0.1453 0.0588 1.0000 1.0000

Descriptive statistics by group
group: 0
   vars  n    mean      sd median trimmed     mad  min   max range skew kurtosis     se
X1    1 23 6268.04 2557.22   6565 6145.53 2616.79 2200 11800  9600 0.31    -0.86 533.22
------------------------------------------------------------------------------------------------------------------------
group: 5
   vars  n    mean      sd median trimmed    mad min  max range skew kurtosis     se
```

```
X1    1 22 3927.27 1795.81   3800 3833.33 1482.6 800 7800  7000 0.39    -0.49 382.87
------------------------------------------------------------------------------------------------------------------------
group: 9
   vars  n    mean      sd median trimmed     mad min  max range skew kurtosis     se
X1    1 22 3718.18 1379.66   3400 3766.67 1037.82 400 6200  5800 -0.2    -0.34 294.14
------------------------------------------------------------------------------------------------------------------------
group: 16
   vars  n    mean      sd median trimmed    mad  min   max range  skew kurtosis     se
X1    1 22 6109.09 2310.83   6000 6122.22 2965.2 1600 10600  9000 -0.01    -1.03 492.67
------------------------------------------------------------------------------------------------------------------------
group: 23
   vars  n    mean      sd median trimmed     mad min max range skew kurtosis  se
X1    1 22 5636.36 2115.37   5500 5577.78 3113.46 2800 9600  6800 0.19    -1.42 451
------------------------------------------------------------------------------------------------------------------------
group: 28
   vars  n    mean      sd median trimmed    mad  min  max range skew kurtosis     se
X1    1 22 5936.36 2522.57   5700 5888.89 3706.5 2800 9400  6600 0.12    -1.72 537.81
```

Colony Condition – Food (no. cells/colony/d as food)

```
Call: lm(formula = bc.food$n ~ bc.food$trtmnt + bc.food$bfd, data = bc.food)

Residuals:
   Min     1Q Median     3Q    Max
 -8820  -5139  -1811   5218  10780

Coefficients:
                   Estimate Std. Error t value Pr(>|t|)
(Intercept)         9819.98    1068.64   9.189 9.14e-16 ***
bc.food$trtmntref  -1083.33    1164.66  -0.930   0.3540
bc.food$trtmnttest  -113.89    1257.98  -0.091   0.9280
bc.food$bfd          -88.52      50.45  -1.755   0.0817 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 5706 on 128 degrees of freedom
Multiple R-squared:  0.03097,     Adjusted R-squared:  0.008263
F-statistic: 1.364 on 3 and 128 DF,  p-value: 0.2569

Shapiro-Wilk normality test
W = 0.9173, p-value = 6.093e-07
```

#trtmnt

```
Bartlett test of homogeneity of variances
Bartlett's K-squared = 2.2551, df = 2, p-value = 0.3238

Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 0.83525, df = 2, p-value = 0.6586
```

#bfd

```
Bartlett test of homogeneity of variances
Bartlett's K-squared = 13.451, df = 5, p-value = 0.0195
```

```
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 5.3877, df = 5, p-value = 0.3704

------------------------------------------------------------------------------
```

Colony Strength (no. adult bees/colony/d)

```
lm(formula = bc.adults$n ~ bc.adults$trtmnt + bc.adults$bfd, data = bc.adults)

Residuals:
   Min     1Q Median     3Q    Max
 -4860  -1295   -296   1102   6449

Coefficients:
                     Estimate Std. Error t value Pr(>|t|)
(Intercept)           9196.41     559.63  16.433  < 2e-16 ***
bc.adults$trtmntref   -774.58     609.92  -1.270 0.208836
bc.adults$trtmnttest   119.17     658.78   0.181 0.857045
bc.adults$bfd          102.80      26.42   3.891 0.000246 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 2113 on 62 degrees of freedom
Multiple R-squared:  0.2202,      Adjusted R-squared:  0.1825
F-statistic: 5.836 on 3 and 62 DF,  p-value: 0.001408

Shapiro-Wilk normality test
W = 0.98806, p-value = 0.7794
```

#trtmnt

```
Bartlett test of homogeneity of variances
Bartlett's K-squared = 6.1212, df = 2, p-value = 0.04686

Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 1.0833, df = 2, p-value = 0.5818
```

#bfd

```
Bartlett test of homogeneity of variances
Bartlett's K-squared = 17.677, df = 5, p-value = 0.00338

Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 32.988, df = 5, p-value = 3.783e-06

Pairwise comparisons using Wilcoxon rank sum test
data:  bc.adults$n and bc.adults$bfd

   0       5      9      16     23
5  1.0000  -      -      -      -
9  0.0173  0.0034 -      -      -
16 8.5e-05 0.0011 0.0247 -      -
23 0.2708  0.0633 1.0000 0.2783 -
28 0.1003  0.0384 1.0000 0.2352 1.0000

P value adjustment method: holm
```

```
Descriptive statistics by group
group: 0
vars  n    mean     sd median trimmed     mad  min   max range skew kurtosis     se
X1    1 11 8574.09 985.97   8515 8551.11 1060.06 7150 10205  3055 0.07    -1.46 297.28
------------------------------------------------------------------------------------------------------------------------------------------------------
group: 5
vars  n    mean     sd median trimmed    mad  min  max range  skew kurtosis     se
X1    1 11 8284.55 851.16   8385 8327.22 481.84 6695 9490  2795 -0.44    -0.88 256.63
------------------------------------------------------------------------------------------------------------------------------------------------------
group: 9
vars  n     mean      sd median  trimmed    mad  min   max range skew kurtosis     se
X1    1 11 10405.91 1288.07  10075 10298.89 578.21 8515 13260  4745 0.84  -0.08 388.37
------------------------------------------------------------------------------------------------------------------------------------------------------
group: 16
vars  n     mean      sd median trimmed    mad   min   max range skew kurtosis     se
X1    1 11 13070.91 1958.53  12935   12935 1541.9 10075 17290  7215 0.53  -0.36 590.52
------------------------------------------------------------------------------------------------------------------------------------------------------
group: 23
vars  n     mean      sd median  trimmed     mad  min   max range skew kurtosis     se
X1    1 11 10837.27 2563.19   9880 10782.78 3083.81 7085 15080  7995 0.19 -1.55 772.83
------------------------------------------------------------------------------------------------------------------------------------------------------
group: 28
vars  n     mean      sd median  trimmed     mad  min   max range  skew kurtosis    se
X1    1 11 10837.27 2213.65  10205 10883.89 2891.07 7215 14040  6825 -0.05-1.58 667.44
--------------------------------------------------------------------------------
```

Colony Strength (no. juveniles/colony/d)

```
lm(formula = bc.juv$n ~ bc.juv$trtmnt + bc.juv$bfd, data = bc.juv)

Residuals:
    Min      1Q  Median      3Q     Max
-8365.0 -1803.7  -278.9  1933.6  8377.7

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)        13850.6      813.4  17.029  < 2e-16 ***
bc.juv$trtmntref   -1075.0      886.5  -1.213 0.229850
bc.juv$trtmnttest   1538.9      957.5   1.607 0.113088
bc.juv$bfd          -150.7       38.4  -3.924 0.000221 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 3071 on 62 degrees of freedom
Multiple R-squared:  0.2693,      Adjusted R-squared:  0.234
F-statistic: 7.618 on 3 and 62 DF,  p-value: 0.0002048

Shapiro-Wilk normality test
W = 0.98578, p-value = 0.6526

#trtmnt
Bartlett test of homogeneity of variances
Bartlett's K-squared = 12.663, df = 2, p-value = 0.00178

Kruskal-Wallis rank sum test
```

```
Kruskal-Wallis chi-squared = 7.345, df = 2, p-value = 0.02541

Pairwise comparisons using Wilcoxon rank sum test

     cont  ref
ref  0.235 -
test 0.134 0.034

Descriptive statistics by group
group: cont
vars  n     mean      sd median trimmed    mad  min   max range skew kurtosis    se
X1    1 24 11816.67 2700.67  11600   11830 2965.2 6400 16800 10400 0.03   -0.85 551.27
------------------------------------------------------------------------------------------------------------------------------------------------------
group: ref
vars  n     mean      sd median trimmed     mad  min   max range skew kurtosis     se
X1    1 24 10741.67 4595.45  10400   10610 4744.32 2000 20400 18400 0.26  -0.63 938.04
------------------------------------------------------------------------------------------------------------------------------------------------------
group: test
vars  n     mean      sd median trimmed     mad   min   max range skew kurtosis     se
X1    1 18 13355.56 2119.44  13300   13300 2520.42 10400 17200  6800 0.15 -1.36 499.56

#bfd
Bartlett test of homogeneity of variances
Bartlett's K-squared = 2.8835, df = 5, p-value = 0.7179

            Df    Sum Sq  Mean Sq F value  Pr(>F)
bc.juv$bfd   5 441910909 88382182    14.8 1.9e-09 ***
Residuals   60 358232727  5970545

Tukey multiple comparisons of means - 95% family-wise confidence level

Fit: aov(formula = bc.juv$n ~ bc.juv$bfd)
             diff         lwr        upr     p adj
5-0     963.63636  -2103.5054  4030.7781 0.9385470
9-0   -2400.00000  -5467.1418   667.1418 0.2088326
16-0  -6872.72727  -9939.8690 -3805.5855 0.0000002 ***
23-0  -4163.63636  -7230.7781 -1096.4946 0.0023627 **
28-0  -2309.09091  -5376.2327   758.0509 0.2457580
9-5   -3363.63636  -6430.7781  -296.4946 0.0235996 *
16-5  -7836.36364 -10903.5054 -4769.2219 0.0000000 ***
23-5  -5127.27273  -8194.4145 -2060.1310 0.0001000 ***
28-5  -3272.72727  -6339.8690  -205.5855 0.0299073 *
16-9  -4472.72727  -7539.8690 -1405.5855 0.0008902 ***
23-9  -1763.63636  -4830.7781  1303.5054 0.5418982
28-9     90.90909  -2976.2327  3158.0509 0.9999993
23-16  2709.09091   -358.0509  5776.2327 0.1130718
28-16  4563.63636   1496.4946  7630.7781 0.0006630 ***
28-23  1854.54545  -1212.5963  4921.6872 0.4861431

--------------------------------------------------------------------------------
Brood Index (bi)
Call: lm(formula = value ~ group.phase + group.trtmnt, data = indices)
```

```
Residuals:
     Min       1Q   Median       3Q      Max
-1.53830 -0.52615 -0.02025  0.44034  1.79170

Coefficients:
                 Estimate Std. Error t value Pr(>|t|)
(Intercept)        2.2826     0.2831   8.064 6.47e-10 ***
group.phasemon     0.8482     0.2726   3.111  0.00343 **
group.trtmntref   -1.5725     0.2768  -5.680 1.33e-06 ***
group.trtmnttest   0.2171     0.2990   0.726  0.47207
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.783 on 40 degrees of freedom
Multiple R-squared:  0.5845,      Adjusted R-squared:  0.5534
F-statistic: 18.76 on 3 and 40 DF,  p-value: 9.385e-08

Shapiro-Wilk normality test
W = 0.96586, p-value = 0.2146

Bartlett test of homogeneity of variances
Bartlett's K-squared = 3.0852, df = 2, p-value = 0.213
```

Exposure Period (BFD 5)

```
               Df  Sum Sq    Mean Sq  F value   Pr(>F)
   group        2   4.724     2.3618   7.659     0.0139 *
   Residuals    8   2.467     0.3084

   Multiple Comparisons of Means: Dunnett Contrasts
                            Estimate Std. Error t value    Pr(>|t|)
   ref - cont == 0   -1.3125     0.3927  -3.342      0.0186 *
   test - cont == 0   0.1092     0.4241   0.257      0.9540
```

Monitoring Period

```
               Df      Sum Sq   Mean Sq F value   Pr(>F)
   group        2      13.84    6.919   11.59     0.000512 ***
   Residuals   19      11.34    0.597

   Multiple Comparisons of Means: Dunnett Contrasts
                     Estimate Std. Error t value   Pr(>|t|)
   ref - cont == 0     -1.5188  0.3863     -3.932    0.00173 **
   test - cont == 0    0.2688   0.4172      0.644    0.75143
```

BFD 9

```
                  Df Sum Sq Mean Sq F value Pr(>F)
      group        2  7.019  3.510   4.785   0.043 *
      Residuals    8  5.868  0.733

      Multiple Comparisons of Means: Dunnett Contrasts
                       Estimate Std. Error t value  Pr(>|t|)
      ref - cont == 0   -1.4750  0.6056     -2.436   0.0724
      test - cont == 0  0.3683   0.6541      0.563   0.8052
```

BFD 16

```
                   Df Sum Sq Mean Sq F value   Pr(>F)
      group         2  6.854  3.427   5.2       0.0357 *
```

```
       Residuals     8 5.272   0.659

       Multiple Comparisons of Means: Dunnett Contrasts
                          Estimate Std. Error t value  Pr(>|t|)
       ref - cont ==   0  -1.5625  0.5740     -2.722   0.0469 *
       test - cont == 0   0.1692   0.6200     0.273    0.9486
```

BFD 23

```
                   Df Sum Sq Mean Sq F value  Pr(>F)
       group        2  10.63  5.313   5.133    0.0368 *
       Residuals    8  8.28   1.035

       Multiple Comparisons of Means: Dunnett Contrasts
                         Estimate Std. Error t value  Pr(>|t|)
       ref - cont == 0   -1.9400  0.7194     -2.697   0.0487 *
       test - cont == 0  0.2217   0.7770      0.285   0.9440
```

Brood Compensation Index (bci)

```
Call: lm(formula = value ~ group.phase + group.trtmnt, data = indices)

Residuals:
     Min       1Q   Median       3Q      Max
-2.10489 -0.45804  0.07968  0.47219  1.99511

Coefficients:
                 Estimate Std. Error t value Pr(>|t|)
(Intercept)        2.2203     0.2972   7.471 4.18e-09 ***
group.phasemon     1.2045     0.2862   4.208 0.000141 ***
group.trtmntref   -1.2600     0.2907  -4.335 9.57e-05 ***
group.trtmnttest   0.1754     0.3140   0.559 0.579459
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.8221 on 40 degrees of freedom
Multiple R-squared:  0.5287,      Adjusted R-squared:  0.4934
F-statistic: 14.96 on 3 and 40 DF,  p-value: 1.114e-06

Shapiro-Wilk normality test
W = 0.97142, p-value = 0.3393

Bartlett test of homogeneity of variances
Bartlett's K-squared = 3.1317, df = 2, p-value = 0.2089
```

Exposure Period (BFD 5)

```
                Df Sum Sq Mean Sq F value Pr(>F)
   group        2  4.450  2.2251  7.322   0.0156 *
   Residuals    8  2.431  0.3039

   Multiple Comparisons of Means: Dunnett Contrasts
                     Estimate  Std. Error t value Pr(>|t|)
   ref - cont == 0   -1.28000   0.38981     -3.284   0.0203 *
   test - cont == 0  0.09333    0.42105     0.222    0.9656
```

Monitoring Period

```
                Df Sum Sq Mean Sq F value    Pr(>F)
group            2  10.82   5.413   8.691   0.00209 **
Residuals       19  11.83   0.623

Multiple Comparisons of Means: Dunnett Contrasts
                  Estimate Std. Error t value  Pr(>|t|)
ref - cont == 0   -1.3525   0.3946     -3.428   0.00539 **
test - cont == 0   0.2204   0.4262      0.517   0.82960
```

BFD 9

```
                Df Sum Sq Mean Sq F value   Pr(>F)
group            2  6.460   3.230   4.506   0.0489 *
Residuals        8  5.734   0.717

Multiple Comparisons of Means: Dunnett Contrasts
                    Estimate Std. Error t value Pr(>|t|)
ref - cont == 0     -1.4150   0.5987     -2.364  0.0808
test - cont == 0    0.3533    0.6466      0.546  0.8150
```

BFD 16

```
                Df Sum Sq Mean Sq F value Pr(>F)
group            2  4.499   2.2494  3.017   0.106
Residuals        8  5.965   0.7456
```

BFD 23 (24 DAT)

```
               Df Sum Sq Mean Sq F value Pr(>F)
group           2  3.280   1.640   3.98    0.0631
Residuals       8  3.296   0.412
```

Brood Termination Rate (btr, %)

```
Call: lm(formula = value ~ group.phase + group.trtmnt, data = indices)

Residuals:
    Min      1Q  Median      3Q     Max
-35.354 -14.584   5.994  10.017  39.826

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)       19.962      6.775   2.947  0.00534 **
group.phasemon     6.949      6.525   1.065  0.29322
group.trtmntref   38.793      6.626   5.855 7.56e-07 ***
group.trtmnttest  -5.690      7.157  -0.795  0.43130
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 18.74 on 40 degrees of freedom
Multiple R-squared:  0.5607,      Adjusted R-squared:  0.5277
F-statistic: 17.02 on 3 and 40 DF,  p-value: 2.81e-07

Shapiro-Wilk normality test
W = 0.94794, p-value = 0.04616

Bartlett test of homogeneity of variances
```

Bartlett's K-squared = 11.331, df = 2,
p-value = 0.003463

Exposure Period (BFD 5)

Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 5.3258, df = 2,
p-value = 0.06975

Monitoring Period

Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 10.321, df = 2, p-value = **0.00574**

Wilcoxon rank sum test with continuity correction
ref - cont == W = 8, p-value = **0.01345**
test – cont == W = 33.5, p-value = 0.2442

BFD 9

Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 4.7546, df = 2, p-value = 0.0928

BFD 16

Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 4.5985, df = 2, p-value = 0.1003

BFD 23 (24 DAT)

Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 3.9621, df = 2, p-value = 0.1379